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Gene Expression Profiling of Host Response in Models of Acute HIV Infection[1]

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HIV infection is characterized by a host response composed of adaptive and innate immunity that partially limits viral replication; however, it ultimately fails in eradicating the virus. To model host gene expression during acute HIV infection, we infected cynomolgus macaques with the SIV/HIV-1 chimeric virus, SHIV89.6P, and profiled gene expression in peripheral blood over a 5-wk period using a high density cDNA microarray. We demonstrate that viral challenge induced a widespread suppression of genes regulating innate immunity, including the LPS receptors, CD14 and TLR4. An overexpression of 16 IFN-stimulated genes was also observed in response to infection; however, it did not correlate with control over viral titers. A statistical analysis of the dataset identified 10 genes regulating apoptosis with differential expression during the first 2 wk of infection ($p < 0.004$). Quantitative real-time PCR verified transcriptional increases in IFN-α-inducible genes and decreases in genes regulating innate immunity. Therefore, the persistence of high viral loads despite an extensive IFN response suggests that HIV can resist in vivo IFN treatment despite published reports of in vitro efficacy. The transcriptional suppression of genes regulating innate immunity may allow HIV to evade acute host responses and establish a chronic infection and may reduce innate host defense against opportunistic infections. *The Journal of Immunology,* **2004, 173: 6858–6863.**

Infection by HIV typically results in a chronic disease whereby $CD4^+$ T cells are gradually depleted and host immunity becomes increasingly impaired. The ability of HIV to evade the adaptive response in the long term has been attributed to diverse mechanisms, most importantly the emergence of mutant variants that avoid neutralizing Abs and CTL responses, and also the loss and dysfunction of HIV-specific $CD4^+$ T cells, down-regulation of class I HLA molecules on infected cells, and defective Ag responses of $CD8^+$ cells (1). Early host responses against HIV are predominately mediated by the $CD8^+$ killing of virus-infected cells. Transient disruption of CTL activity during acute infection of macaques results in higher viral loads and faster progression to clinical disease than control animals, demonstrating the importance of the early response in establishing the impact of the chronic phase of the disease (2).

During infection, HIV drives a vast program of host cell RNA expression, an activity attributed predominately to the product of the viral *nef* gene (3). Humans and macaques infected with *nef*-deficient viruses generally have low viral loads and exhibit mild, if any, effects on immune function (4). Similarly, the product of the *tat* gene has been demonstrated to drive extensive host gene expression in vitro (5, 6). Reduction of tat activity by tat toxoid vaccination in nonhuman primates reduced disease severity that correlated with reduced IFN-α expression (7). We hypothesized that aberrant host expression during acute infection with a wild-type virus may be responsible for the evasion of host immunity. We approached this question by analyzing the genes expressed during acute infection of cynomolgus macaques with an SIV/HIV chimeric virus (SHIV).[3] In this study, we demonstrate that acute infection induces a marked decrease in genes regulating innate immunity that correlates inversely with levels of viral replication. Further, we characterize an extensive type I IFN response with no apparent control over viral replication, and identify apoptosis-regulating genes with differential transcription during infection.

Materials and Methods

Virus

SHIV89.6P was kindly provided by Dr. K. Reimann (Harvard University Medical School, Boston, MA). The parental virus, SHIV89.6, was constructed using SIVmac239 core (*gag*, *pol*, *vif*, *vpx*, *vpr,* and *nef*), HIV-1 auxiliary genes (*tat*, *rev*, and *vpu*), and *env* from an HIV-1 cytopathic primary isolate, 89.6 (8). The added virulence of SHIV89.6P was attained by serial passage through four rhesus monkeys (*Macaca mulatta*; Ref. 9).

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[3] Abbreviations used in this paper: SHIV, SIV/HIV chimeric virus; QRT-PCR, quantitative RT-PCR; PI, post infection; MX1, myxovirus resistance 1; ISG, IFN-stimulated genes; ALOX5, arachidonate 5-lipoxygenase; ALOX5AP, arachidonate 5-lipoxygenase activating protein; PLAUR, urokinase-type plasminogen activator receptor; ORM2, orosomucoid 2; BCL, B cell line; BAG, BCL2-associated athanogene.

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Animals and infections

Four juvenile cynomolgus macaques were inoculated by the i.v. route with 10 macaque ID_{50} of SHIV89.6P at the onset of the study. All animals used in this study were colony bred within the Non-Human Primate Breeding Colony of Health Canada under the Canadian Council of Animal Care approved conditions. The animal experimentation was approved by the Animal Care Committee of Health Canada, which is accredited by the Canadian Council of Animal Care. All animals were serologically negative for the herpes B virus, simian T cell lymphotropic virus-1, simian retrovirus-1, -2, and -5, and SIV. Animals 071, 115, and 067, 015 were euthanized humanely at 4 and 5 wk postinfection (PI), respectively, in accordance with Canadian Council of Animal Care regulations.

Cytometry

The percentage of $CD4^+$ T lymphocytes was determined using a FACScan flow cytometer and CellQuest software (BD Biosciences, San Jose, CA). Whole blood collected in EDTA was analyzed for lymphocyte subsets by incubation with FITC-labeled antihuman CD2 and CD4 PE-labeled antihuman (BD Biosciences). White blood cell counts were obtained from a Coulter Counter S-PLUS IV hematology workstation (Beckman Coulter, Fullerton, CA) and were used to calculate the lymphocyte subset absolute counts.

Plasma virus RNA load

Quantitative assays for SHIV RNA were performed by Bayer (Emeryville, CA), using a branched DNA signal amplification method similar to the Quantiplex HIV-RNA-branched DNA. Target probes designed to hybridize with the *pol* region of the SIVmac group of strains were used. The results were quantified by comparison with purified and quantitated in vitro-transcribed SIV*pol* RNA. Assay sensitivity was 500 copies of SIV RNA per milliliter.

RNA isolation, amplification, and hybridization

A total of 2.5 ml of whole blood was drawn directly into 2.5-ml PAXgene blood RNA tubes, and total RNA was purified with PAXgene blood RNA kits (Qiagen, Valencia, CA). Per sample, 2 μg of total RNA was amplified using the MessageAMP amplified RNA kit (Ambion, Austin, TX). Probes for microarray hybridization were prepared by labeling 6 μg of amplified RNA with Cy3 or Cy5 by reverse transcription, and hybridization of the labeled cDNA on human 19k3 microarray slides containing 19,008 expressed sequence tags at 37°C for 18 h. Detailed information on the labeling and hybridization procedures can be obtained at http://transnet.uhnres.utoronto.ca, and for the 19k3 microarray at http://microarray.ca.

Microarray data analysis

Microarrays were scanned using a Scanarray Express Scanner (Packard Bioscience, Boston, MA) at 10-μm resolution. QuantArray version 3.0 software (Packard Bioscience) was used to quantitate slide images. Individual slide data was background subtracted and normalized by global intensity median using the QuantArray normalization Excel macro. Eight measurements of gene expression per interval were obtained for each animal. Data from biological replicates at each time point were combined, outliers were removed, and genes were subjected to a *t* test with a nominal *p* value of 0.05 using ArrayStat v1.0 software (Imaging Research, St. Catharines, Ontario, Canada). To reduce errors of inference, the false discovery rate multiple test correction was used and yielded an effective *p*-value of ~0.02 for each interval dataset. For clustering, the data were filtered to include only genes significantly differential ($p < 0.05$) with corrected mean $\log_{10}$ ratio ≥ 0.301 or ≤ -0.301 at one or more intervals. *k*-means partitional clustering with Euclidean and average linkage distance metrics between the data points and clusters, respectively, was used to organize the filtered dataset into 20 clusters using GeneLinker Platinum version 2.0 software (Molecular Mining, Kingston, Canada). To identify genes with related function, manual searches of the GenBank, OMIM, and LocusLink databases were combined with the ontology search tool, DAVID (10).

Quantitative real-time PCR (QRT-PCR)

QRT-PCR was performed on total RNA using an ABI-PRISM 7900HT Sequence Detection System and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). A total of 250 ng of total RNA was reverse transcribed in 20 μl of reaction under the following conditions: 6.25 μM dN6 random hexanucleotide primer (Applied Biosystems), 50 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 75 mM KCl, 500 μM dATP, dGTP, dTTP, dithiothreitol and dCTP, 10 mM, and 200 U of SuperScript II RNase H^- reverse transcriptase at 42°C for 1 h. Each QRT-PCR was performed in a volume of 25 μl with 0.25-μl cDNA primer pair, and 12.5 μl of SYBR Green PCR Master Mix in ABI-PRISM optical 96-well plates. Primer pairs were designed to generate intron-spanning products of ~100 bp using Primer Express version 2.0 software (Applied Biosytstems) or were deduced from literature. Each primer pair was tested with a logarithmic dilution of cDNA to generate a standard curve, which was used to calculate the starting quantity of target RNA. Primers specific for 18s-rRNA was used as an endogenous standard to normalize samples. Fold-change was calculated by dividing the normalized postinfected sample quantity with the normalized preinfected control quantity. The 5′-3′ sequences of primer pairs: myxovirus resistance 1 (MX1), forward, AGG AGT TGC CCT TCC CAG A, reverse, TCG TTC ACA AGT TTC TTC AGT TTC A (11); CXCL10, forward, TCC ACG TGT TGA GAT CAT TGC, reverse, TCT TGA TGG CCT TCG ATT CTG; TLR4, forward, CAG AGT TTC CTG CAA TGG ATC A, reverse, GCT TAT CTG AAG GTG TTG CAC AT, (12); CD14, forward, CGC TCC GAG ATG CAT GTG, reverse, TTG GCT GGC AGT CCT TTA GG (12); IL-1R1, forward, GCT GGC TGG GTG GTT CAT, reverse, TCC AGC TCA AGC AGG ACA ACT; IL-1RN, forward, CCG ACC CTC TGG GAG AAA AT, reverse, TGG TTG TTC CTC AGA TAG AAG GTC TT; 18s-rRNA, forward, CGG CTA CCA CAT CCA AGG AA, reverse, GCT GGA ATT ACC GCG GCT.

Results

To model acute in vivo HIV infection, we infected cynomolgus macaques (*Macaca fascicularis*) i.v. with SHIV89.6P, serially passaged in vivo for enhanced virulence (8, 9). Consistent with published reports (8, 9) and our own previous results (data not shown), the $CD4^+$ population permanently decreased below preinfected levels by 2 wk PI (Fig. 1*a*). Similarly, plasma virus levels rose sharply after infection, peaking at 2 wk, and remained elevated until euthansasia (Fig. 1*b*).

We examined the molecular changes associated with in vivo SHIV infection by microarray analysis of RNA from peripheral

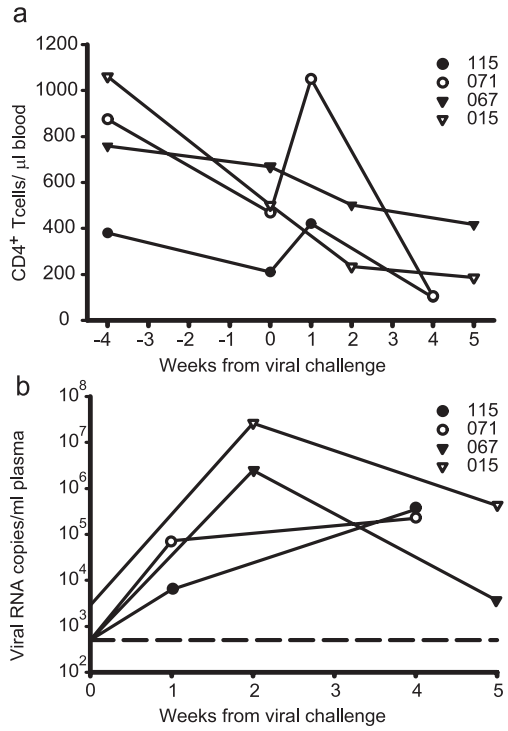

FIGURE 1. Virologic and immunologic monitoring in SHIV89.6P-infected macaques. Cynomolgus macaques were i.v. infected with SHIV89.6P and were monitored for changes in the levels of peripheral $CD4^+$ T cells (*a*) and plasma SIV RNA (*b*). Animals 115 and 071 were monitored at 0, 1, and 4 wk PI, and animals 067 and 015 at 0, 2, and 5 wk PI. The dashed line in *b* indicates the assay sensitivity limit (500 copies/ml). The viral load of animal 015 was detected above threshold before viral challenge at week 0 by a false-positive error of the assay; this animal was determined to be virus-free by ELISA previous to infection.

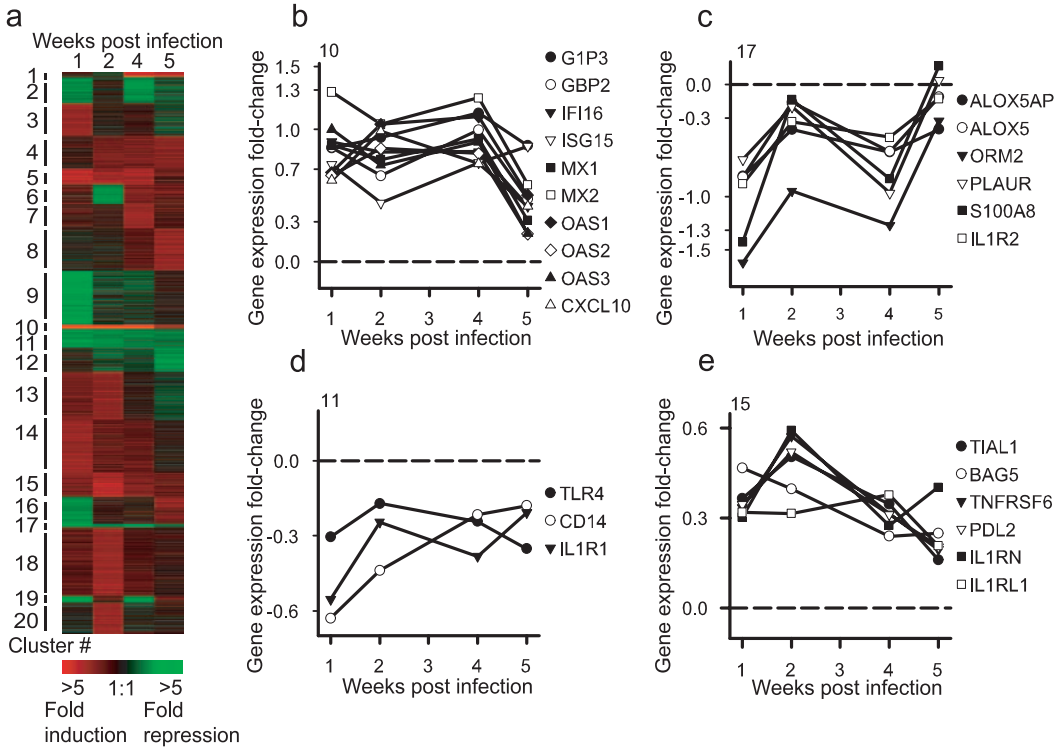

FIGURE 2. Gene expression induced by SHIV89.6P infection. *a*, Expression profiles of peripheral blood genes whose RNA levels were affected significantly by SHIV89.6P infection were organized into groups sharing similar kinetics and magnitude using *k*-means clustering ($k = 20$). The fold-change in expression levels is relative to preinfected samples and is displayed in red (increased expression) or green (decreased expression). *b–e*, Fold-change (on a $\log_{10}$ scale) in expression of genes within individual clusters. in *a*, the dotted line represents no fold-change in expression ($\log_{10} = 0$), the number in the *top left corner* notes the representative cluster in *a*. Expression levels are the mean values measured across all animals. *b*, Cluster 10, containing IFN-inducible genes. *c*, Cluster 17, containing innate immune response genes. *d*, Cluster 11, containing Toll/IL-1R genes. *e,* Cluster 15, containing apoptosis regulators.

whole blood samples from 1-, 2-, 4-, and 5-wk intervals, following infection competitively hybridized against preinfected samples using a 19,008 element cDNA microarray. We identified 2406 genes significantly differential ($p < 0.025$) in one or more intervals (complete dataset available at the GEO microarray data repository, www.ncbi.nlm.nih.gov/geo/, accession number GSE1854). We used a partitional clustering algorithm to organize differentially expressed genes into highly reproducible groups sharing similar expression kinetics (Fig. 2*a*). The genes exhibiting the greatest increase in relative expression were grouped within a single cluster; the majority of these genes were type I IFN-stimulated genes (ISGs; Fig. 2, *a* (cluster 10) and *b*). cDNAs demonstrating the greatest decrease in relative abundance were assembled in a single cluster: these genes were chiefly regulators of innate resistance to bacteria: arachidonate 5-lipoxygenase (ALOX5), its activating protein, arachidonate 5-lipoxygenase activating protein (ALOX5AP), the urokinase-type plasminogen activator receptor (PLAUR), the type II receptor for IL-1, IL-1R2 (Fig 2, *a* (cluster 17) and *c*), and two inflammatory serum proteins, orosomucoid 2 (ORM2) and S100A8 (Fig. 2*c*). Three cDNAs central to the Toll/IL-1 pathway regulation of innate inflammatory responses, CD14, TLR4, and type I receptor for IL-1, IL-1R1, demonstrated decreased expression (Fig. 2, *a* (cluster 11) and *d*). Conversely, two genes antagonistic to Toll/IL-1R signaling, the IL-1R antagonist, IL-1RN and the IL-1R-like protein 1, were observed to be up-regulated at 2 wk PI (Fig. 2, *a* (cluster 15) and *e*), coinciding with the peak of $CD4^+$ depletion and plasma virus levels (Fig. 1, *a* and *b*). Within the same cluster were four genes regulating apoptosis: TNFRSF6/FAS, programmed death ligand 2, cytotoxic granule-associated RNA-binding protein-like 1, and B cell line (BCL) 2-associated athanogene (BAG)5 (Fig. 2, *a* (cluster 15) and *e*). The arrangement of cDNAs

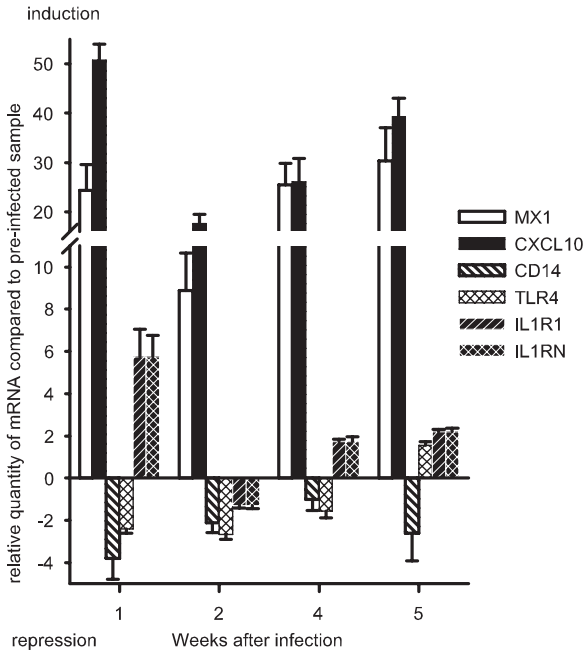

FIGURE 3. Real-time PCR analysis of gene expression induced by SHIV89.6P. Real-time PCR of select SHIV responsive genes. The *y*-axis indicates the relative quantity of starting specific mRNA in the sample as compared with the preinfected sample. The results were normalized to the level of endogenous 18s-rRNA. Error bars indicate the product of the SD of the relative quantities and the linear fold-change.

with related function within individual clusters indicates that the clustering algorithm is effectively identifying groups of coregulated genes. To verify the changes in transcript abundance observed in the microarray analysis, we analyzed six genes, *MX1*, *CXCL10*, *CD14*, *TLR4*, *IL-1R1*, and *IL-1RN* using QRT-PCR (Fig. 3). The polarity and magnitude of the fold-change for each cDNA matched well with the measurements from the microarray data.

We classified genes into functional categories using the LocusLink database (www.ncbi.nlm.nih.gov/locuslink) in combination with publicly available ontology tools (Table I). Ontology classification also highlighted early increases in expression of the anti-apoptosis protein BCL2 and several BCL2-related proteins: BCL2L1, BCL11b, BAG3, and BAG5.

Discussion

In this study, we have used microarray analysis to identify host genes regulated during the acute phase of SHIV89.6P infection in vivo. During SHIV replication, we observed a reduced expression of both type I and II IL-1 receptors, CD14, and TLR4, all components of the Toll-like family of molecules that regulate innate immunity. Recent evidence has demonstrated that Toll receptors regulate the ability of dendritic cells to activate HIV-1 *gag*- and *nef*-specific $CD8^+$ T cells from HIV^+ donors (13). Disruption of Toll immunity by HIV has recently been reported; directed expression of the HIV-1 accessory protein, Vpu, inhibited activation of the drosophila Toll pathway, which is highly homologous to mammalian Toll signaling, and *vpu* transgenic files had increased susceptibility to fungal infections (14). Stimulation of the TLR2, TLR4, and TLR9 receptors in vitro have been demonstrated to induce transcription from the HIV long terminal repeat via NF-κB activation, and reducing Toll activation by ligand tolerization decreased the production of IL-1, TNF-α, and IL-6, while viral replication increased compared with nontolerized samples (15). Thus, the reduced expression of CD14, TLR4, and IL-1R during acute SHIV infection may inhibit activation of virus-specific CTLs, the most important component of early immunity to SIV and HIV. Further studies directly measuring direct CTL activity during acute infection will be required to test this hypothesis. Down-regulating expression of CD14 and TLR4 may also impair host immunity against opportunistic infections. In addition to Gram-negative

Table 1. *Genes differentially expressed in response to SHIV89.6P infection*

GO Symbol	Description	Unigene	Gene Expression Fold-Change Weeks PI[a]			
			1	2	4	5
	IFN-inducible genes					
MX1	Myxovirus (influenza) resistance 1	Hs.76391	**7.8**	**7.1**	**11.0**	**2.0**
MX2	Myxovirus (influenza) resistance 2	Hs.926	**16.2**	**8.6**	**16.5**	**4.6**
G1P3	IFNα-inducible protein	Hs.265827	**11.6**	**9.9**	**9.5**	**5.0**
OAS1	2′,5′-oligoadenylate synthetase 1 (40–46 kD)	Hs.82396	**4.9**	**17.0**	**11.3**	**2.3**
OAS2	2′-5′-oligoadenylate synthetase 2 (69–71 kD)	Hs.264981	**4.5**	**7.2**	**6.6**	1.6
OAS3	2′-5′-oligoadenylate synthetase 3 (100 kD)	Hs.56009	**7.6**	**4.4**	**8.7**	2.0
GBP2	Guanylate binding protein 2	Hs.171862	**8.5**	**5.2**	**7.7**	**2.4**
IFRG28	28 kD IFN responsive protein	Hs.43388	**4.8**	**2.0**	**2.2**	**2.6**
IFIT1	IFN-Induced protein with tetratricopeptide repeats 1	Hs.20315	**6.5**	1.0	**1.4**	**2.6**
IFITM1	IFN-induced transmembrane protein 1	Hs.146360	**2.2**	**2.4**	**1.5**	**1.7**
ISG20	IFN-stimulated gene (20 kD)	Hs.183487	**3.6**	**1.7**	**2.0**	**1.5**
ISG15	IFN-stimulated protein (15 kD)	Hs.833	**3.8**	1.1	**2.5**	**4.9**
STAT1	Signal transducer and activator of transcription 1 (91 kD)	Hs.21486	**3.4**	**2.6**	**2.6**	**1.6**
CXCL10	Small inducible cytokine subfamily B (Cys-X-Cys), member 10	Hs.2248	**2.8**	**13.9**	**7.3**	**2.3**
PRKRA	Protein kinase, interferon-inducible dsRNA-dependent activator	Hs.18571	**1.9**	**2.7**	1.3	1.5
R158	Retinoic acid and interferon-inducible protein	Hs.27610	**2.4**	**2.7**	**3.3**	**4.5**
	Apoptosis regulators					
TNFRSF6	Tumor necrosis factor receptor superfamily, member 6 (FAS)	Hs.82359	**2.2**	**3.8**	**2.1**	1.6
BCL2	B cell CLL/lymphoma 2	Hs.79241	**2.6**	1.2	1.2	0.6
BAG5	BCL2-associated athanogene 5	Hs.5443	**2.9**	**2.5**	1.7	1.8
BAG3	BCL2-associated athanogene 3	Hs.15259	**1.4**	**1.8**	1.1	**1.3**
BCL11B	B cell CLL/lymphoma 11B	Hs.57987	**2.5**	**1.5**	1.3	0.7
BCL2L1	BCL2-like 1	Hs.305890	1.4	**2.2**	**1.9**	**1.3**
DAD1	Defender against cell death 1	Hs.82890	**2.6**	1.3	1.2	1.2
CUL1	Cullin 1	Hs.14541	**2.7**	**3.4**	**1.5**	1.1
TIAL1	TIA1 cytotoxic granule-associated RNA-binding protein-like 1	Hs.18274	**2.3**	**3.2**	**2.2**	**1.4**
PDL2	Programmed death ligand 2	Hs.61929	**1.4**	**2.8**	**2.0**	**2.0**
	Toll/IL1 pathway					
IL1R2	Interleukin 1 receptor, type II	Hs.25333	**0.1**	**0.3**	**0.2**	**0.4**
IL1RN	Interleukin 1 receptor antagonist	Hs.81134	**2.4**	**4.8**	**1.5**	**1.3**
IL1R1	Interleukin 1 receptor, type I	Hs.82112	**0.3**	**0.6**	**0.4**	**0.6**
IL1RL1	Interleukin 1 receptor-like 1	Hs.66	**2.2**	**1.9**	**1.7**	**1.2**
CD14	CD14 Ag	Hs.75627	**0.2**	**0.3**	**0.4**	**0.4**
TLR4	Toll-like receptor 4	Hs.159239	**0.4**	0.8	**0.6**	**0.7**
	Pulmonary resistance					
PLAUR	Plasminogen activator, urokinase receptor	Hs.179657	**0.6**	0.6	**0.1**	1.0
ALOX5	Arachidonate 5-lipoxygenase	Hs.89499	**0.2**	**0.3**	**0.5**	**0.6**
ALOX5AP	Arachidonate 5-lipoxygenase-activating protein	Hs.100194	**0.1**	**0.3**	**0.2**	**0.5**

[a] Gene expression ratios are as compared to the preinfected transcript levels. Ratios in bold are determined to be statistically significant at $p < 0.025$.

bacteria, CD14 and TLR4 are necessary for protection from CMV and *Candida albicans,* two pathogens prevalent in late stage AIDS patients; however, as our study was focused on the acute phase of infection, further work examining the expression pattern of CD14 and TLR4 during the chronic stage of SHIV infection is required (16, 17).

We also observed changes in the expression of several molecules that mediate innate immunity independently of the TLR/IL-1R machinery, in particular ORM2, ALOX5, ALOX5AP, and PLAUR. ORM2 is a plasma protein that blocks viral entry in vitro. Suppression of ORM2 during replication may aid HIV in dissemination within the host (18). The molecules, ALOX5 and ALOXAP, are the central enzymes responsible for leukotriene synthesis, and they mediate protection from pulmonary infections such as *Klebsiella pneumoniae* (19). Consistent with our data, decreases in leukotriene synthesis have been observed in alveolar macrophages isolated from HIV-1^+ patients, attributed to reduced ALOX5 and ALOX5AP expression (20). Like ALOX5, PLAUR acts in a protective capacity against pulmonary pathogens; $PLAUR^{-/-}$ mice have an increased mortality to *Streptococcus pneumoniae* challenge (21). PLAUR is up-regulated during in vitro HIV-1 infection studies, but down-regulated on peripheral granulocytes from HIV-1-infected patients (22). Treatment with urokinase plasminogen activator, the natural ligand for PLAUR, can block viral replication in vitro (23). Opportunistic infection of the respiratory tract remains an important cause of mortality in HIV-associated disease, particularly in children of resource-poor countries (24). The observed reduction in expression of PLAUR, ALOX5, and ALOX5AP may remove another barrier to viral replication, and impair immunity against pulmonary infection.

We observed a robust induction of ISGs with no correlation with reduction of viral load or stability of peripheral $CD4^+$ levels. Similar results have been reported using macaque infection with SIV, where robust expression of MX1 was detected by QRT-PCR (25). Recent evidence suggests that HIV may orchestrate expression of ISGs to enhance its survival; exogenous CXCL10 treatment enhances the replication of HIV-1 in vitro (26). The absence of an apparent antiviral effect of CXCL10 and other ISGs, despite dramatic increases in their expression, supports a hypothesis whereby the IFN response elicited by in vivo HIV infection may aid replication rather than impede it. Our data document the expression of 16 ISGs to which SHIV infection was resistant during the acute phase (0–5 wk) of infection. This observation addresses a central issue in HIV biology: that SHIV does not suppress the type I response, instead, it is able to persist and replicate at high levels in the midst of high levels of ISG expression. Instead, the virus may evade the type I response at the functional level, indeed, HIV-1 transactivation response RNA-binding protein has been demonstrated to be a potent inhibitor dsRNA PKR activation pathway.

Our microarray data demonstrates that the expression of several apoptotic regulators is altered by SHIV infection, and coincides with the peak of $CD4^+$ death. The activities of FAS and BCL2 within the context of AIDS-associated lymphopenia have been intensely studied. Several data indicate increases in the proportion of FAS^+ T cells within infected patients, accompanied by an increased susceptibility to FAS-mediated apoptosis (27, 28). Ex vivo data has indicated that BCL2 levels are reduced within a subset of $CD8^+$ cells, making them more susceptible to apoptosis (29). Our expression data identifies several novel candidate apoptosis proteins modulated during disease progression.

Recently, studies documenting the transcriptional changes induced by in vivo SIV infection in PBMCs, jejenum biopsies, and frontal lobe sections were published (30–32). In agreement with our findings, increases in the expression of multiple ISGs were observed in all studies. Likewise, elevated expression of ubiquitin-related and proteosomal proteins were observed within infected jejenum and PBMCs at early intervals (2–6 wk for jejenum, 3 wk for PBMC data).

SHIV89.6P is a syncytium-inducing, CXCR4 tropic virus. Recently, it has been suggested that the AIDS-like pathogenesis associated with SHIV89.6P infection in macaques may occur by a different mechanism than that induced by viruses that are CCR5 tropic, such as SIV infecion of macaques or HIV-1 in humans. SHIV89.6P is a widely used model for the preclinical evaluation of AIDS vaccine candidates (33–37). We have used SHIV89.6P to investigate the host response to acute infection by an HIV-like virus. We are currently exploring gene expression profiles associated with HIV pathogenesis using SIV, a more accepted model of AIDS.

In the progression to AIDS, the adaptive immune response declines with the depletion of $CD4^+$ T cells. Our data has demonstrated that SHIV infection induces a marked down-regulation of several key regulators of innate immunity, which may allow the virus to escape host antiviral mechanisms. In the absence of an effective adaptive response, a greater burden would be placed on the innate system, and innate dysfunctions would have a greater impact on host immunity. Therapies designed to reduce the depression of innate immunity would likely have a double-edged effect by limiting viral burden and simultaneously decreasing opportunistic infections.

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